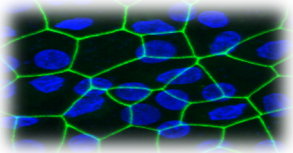
université PARIS-SACLAY

FACULTÉ DE PHARMACIE



Inserm

La science pour la santé
From science to health

ALTERNATIVE METHODS AND THE 3R STRATEGY
WHAT PLACE FOR TISSUE ENGINEERING?

Prof. Saadia Kerdine-Römer

Faculté de Pharmacie, Université Paris Saclay
INSERM UMR-996

ADEBIOTECH, 9 December, Romainville

TISSUE ENGINEERING FOR INDUSTRY 3D reconstructed Tissues and their industrial applications (TEFI)

THE 3RS AND ALTERNATIVE METHODS

Russell and Burch in 1959, originated the concepts of 3R

in 'The Principles of Humane Experimental Technique'

An alternative method allows:

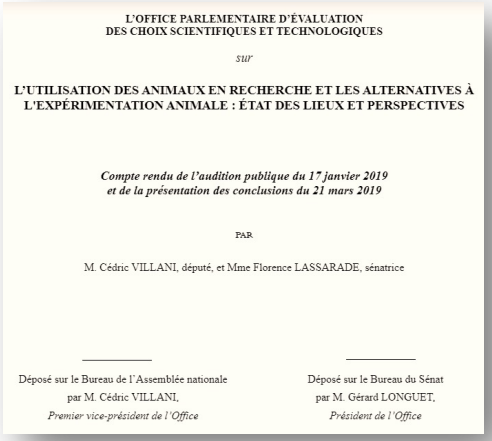
- Replace animal testing
- Reduce the use of animals in specific tests
- Refine a technique to improve animal welfare

Laboratory animals in science is a subject of intense public debate based on legal, moral, and ethical assessments

THE 3RS AND ALTERNATIVE METHODS

The societal context

- Very active animal welfare groups, some of wich are "extremist"
- Press compaigns and political lobbying
- Parliamentary investigations: OPECST report 21 mars 2019



L'OFFICE PARLEMENTAIRE D'ÉVALUATION
DES CHOIX SCIENTIFIQUES ET TECHNOLOGIQUES

sur

L'UTILISATION DES ANIMAUX EN RECHERCHE ET LES ALTERNATIVES À L'EXPÉRIMENTATION ANIMALE : ÉTAT DES LIEUX ET PERSPECTIVES

Compte rendu de l'audition publique du 17 janvier 2019
et de la présentation des conclusions du 21 mars 2019

PAR

M. Cédric VILLANI, député, et Mme Florence LASSARADE, sénatrice

Déposé sur le Bureau de l'Assemblée nationale
par M. Cédric VILLANI,
Premier vice-président de l'Office

Déposé sur le Bureau du Sénat
par M. Gérard LONGUET,
Président de l'Office

"THE USE OF ANIMALS IN RESEARCH AND ALTERNATIVES TO ANIMAL EXPERIMENTATION: CURRENT SITUATION AND PERSPECTIVES"

The regulatory context

Directive 2010/63/EU on the protection of animals used for scientific purposes:

- Applies to all uses (basic, applied research, efficacy and hazard assessment of substances)
- Applies to vertebrate animals, including embryonic forms and cephalopods
- Reinforces the 3Rs principle

Need to develop new alternative methods

Problem of the reliability of the methods already developed

OPECST: Office parlementaire d'évaluation des choix scientifiques et technologiques

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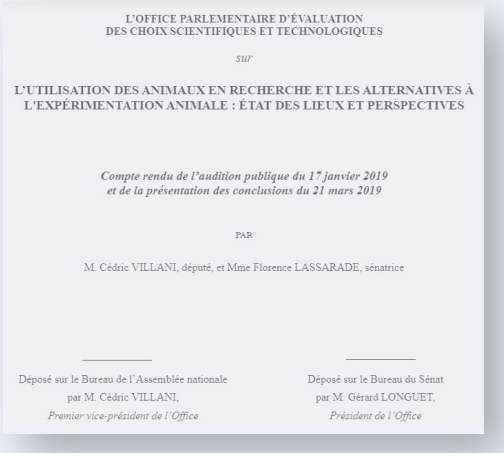

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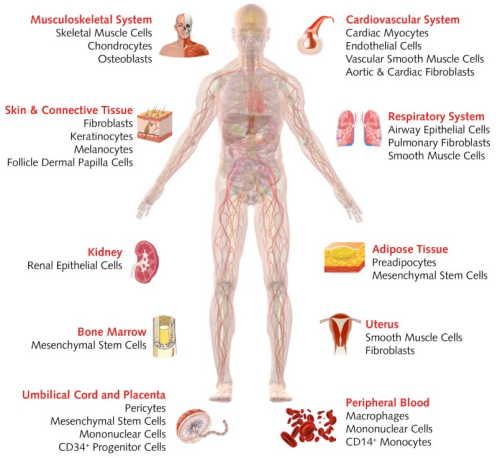
Problem of the reliability of the methods already developed ?

Need to develop new alternative methods and strategy

New **A**pproach **M**ethodologies (NAMs)

CELLS SOURCES & ROLE OF THE MICROENVIRONMENT



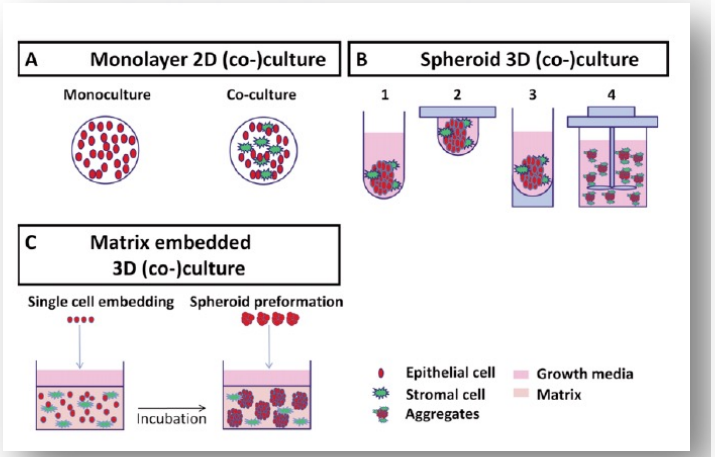
- CELL LINES
- PRIMARY CELLS
- STEM CELLS

- 2D
- 3D

ORGANOIDS

Type	Advantage	Disadvantage
Hydrogel	Tissue like flexibility Easily supplies water-soluble factors to cells	Low mechanical resistance
Solid scaffold	Various materials can be used Physical strength is easily adjusted	Difficulty in homogeneous dispersion of cells
Decellularized native tissue	Provides complex biochemistry, biomechanics and 3D tissues of tissue-specific extracellular matrix (ECM)	Decrease of mechanical properties (roughness, elasticity, and tension strength) of the tissues as compared to the native group
Ultra-low attachment surface	Provides an environment similar to in vivo conditions	Difficulty in mass production Lack of uniformity between spheroids

Cellular Microenvironment



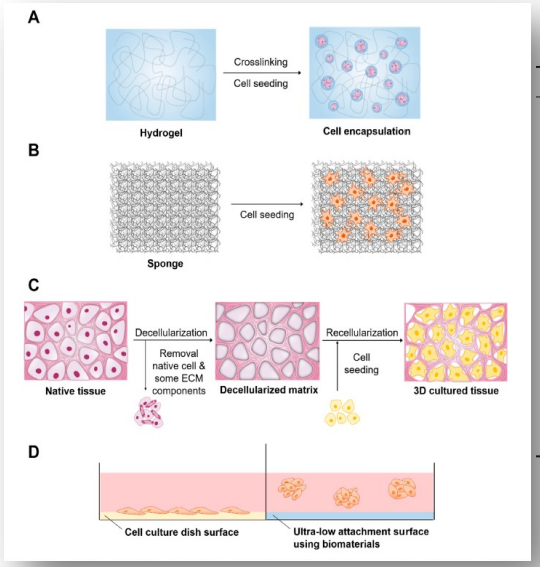
CELLS SOURCES & ROLE OF THE MICROENVIRONMENT

Two main definitions
(CEI, Octobre 2020)

a. The cells self-organise *(1) in* vitro into a 3D structure characteristic of the organ in vivo, (2) the resulting structure is made up of multiple cells present in that particular organ (3) and the cells perform at least some of the functions that they normally perform in that organ.

b. Organoids are 3D structures derived from stem cells or progenitor cells that, at a certain point in time progenitor cells that, on a much smaller scale, recreate important aspects of the 3D anatomy and multicellular repertoire of their physiological counterparts, and can recapitulate basic tissue functions.

ORGANOIDS



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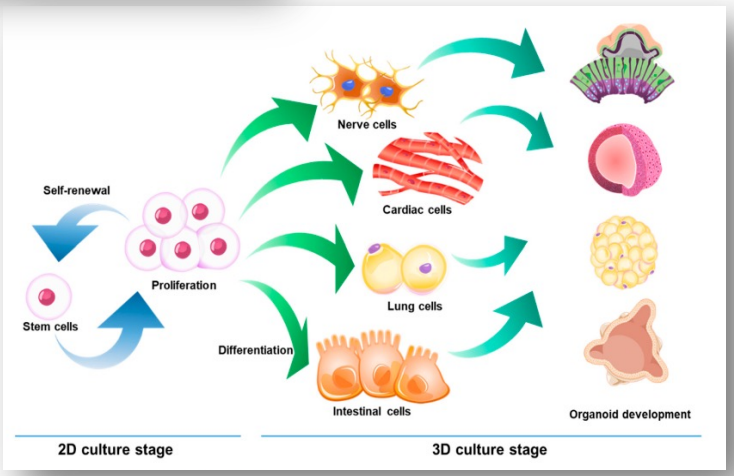
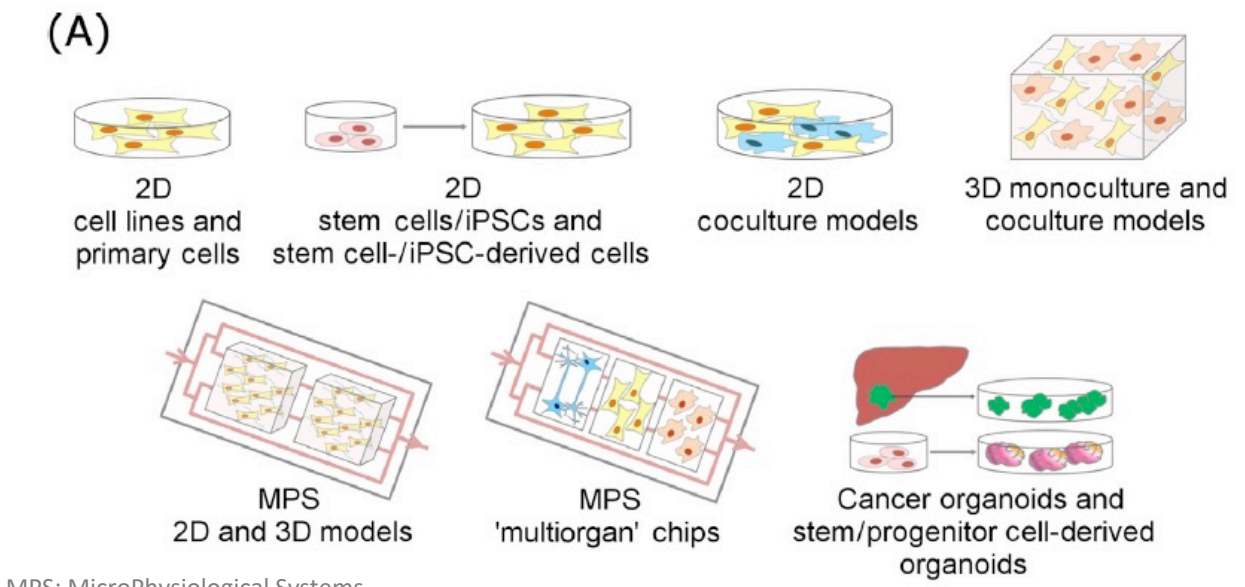
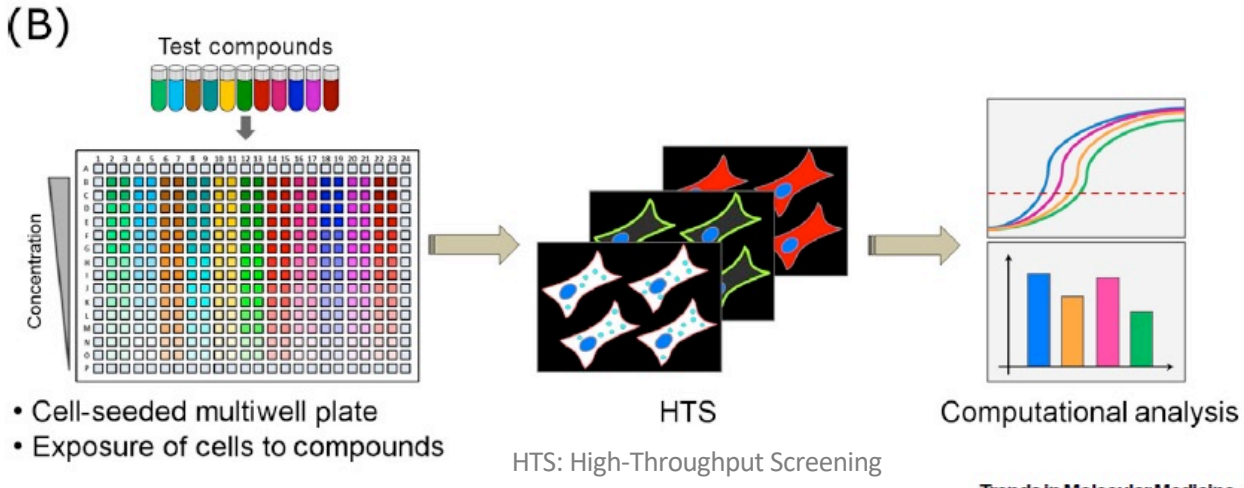
Park *et al., Int. J. Mol. Sci.*, 2021



HUMAN CELL-BASED IN VITRO MODELS



MPS: MicroPhysiological Systems



HTS: High-Throughput Screening

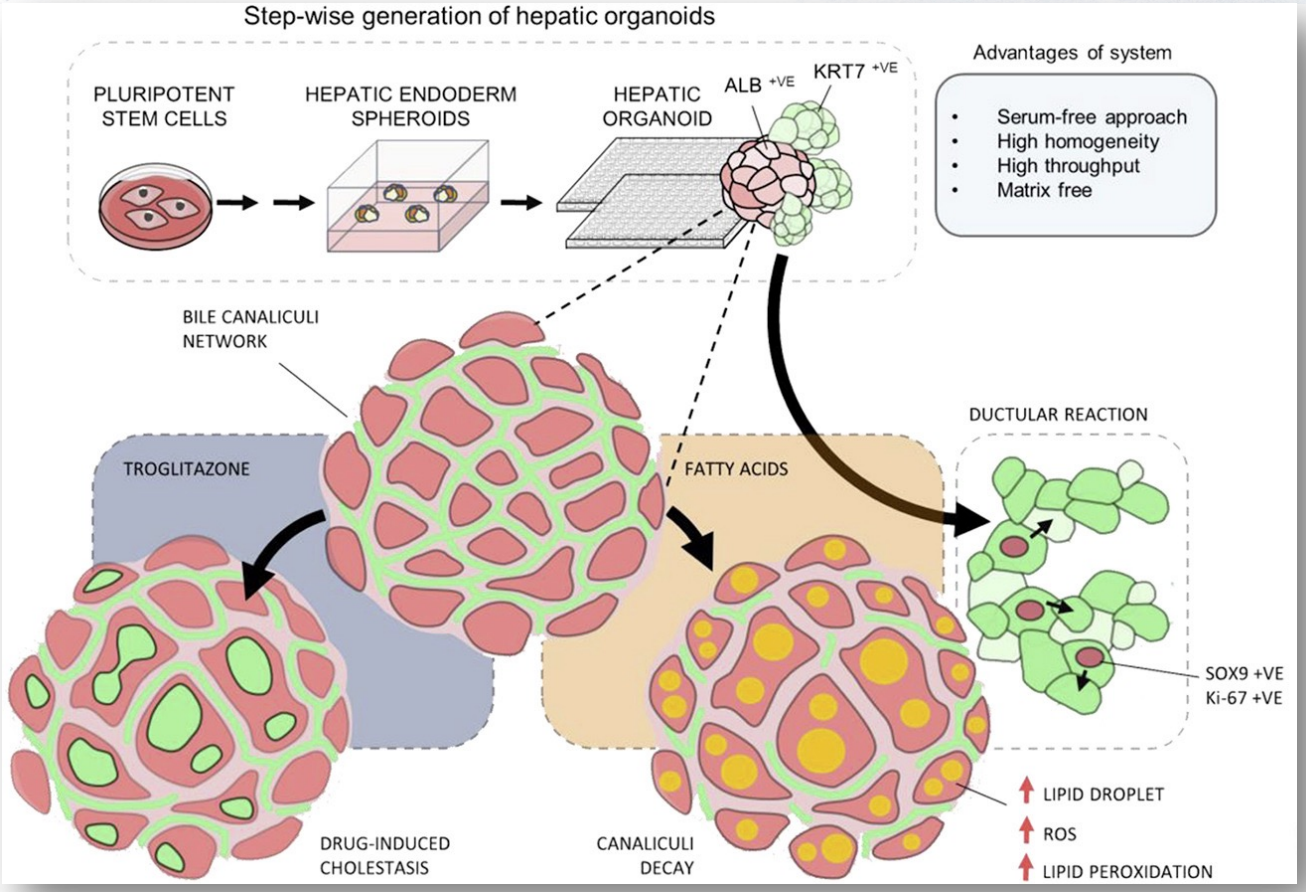
Zink *et al., Trends in Molecular Medicine*, 2020

Advantages and limitations of cell-based in vitro methods

Advantages	Limitations	Potential solutions
Cost effective	No pharmacokinetics/toxicokinetics	Combination with PBPK/TK modeling
Higher/high throughput	Not all mechanisms of toxicity can be covered: limited possibilities to address complex mechanisms of organ damage	More complex organotypic models and use of complementary *in vitro* assays that cover different mechanisms of organ damage
Human cells applicable: eliminates interspecies variability	Limited possibilities to address interactions between different cell types in tissues	Coculture models and iPSC-derived organoids
Suitable for supporting personalized therapies (patient-specific iPSCs and cancer organoids)	Limited possibilities to address organ-to-organ interactions	Multiorgan MPS
Suitable for detailed examination of toxicity mechanisms/easy experimental manipulation	Cellular functions may be altered	Improved cell and cell culture models
	The concentration response of cells may be altered	Improved cell and cell culture models and use of scaling factors in PBPK/TK modeling-based reverse dosimetry

Zink *et al.*, *Trends in Molecular Medicine*, 2020

Exemple: Human pluripotent stem cell-derived organoids as model of liver disease



Bin Ramli *et al.*, Gastroentrology, 2020

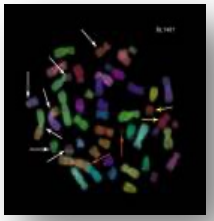
The organoids organized a functional bile canaliculi system, which was disrupted by cholestasis-inducing drugs such as troglitazone.

Incubation of organoids with free fatty acid–enriched media resulted in structural changes associated with nonalcoholic fatty liver disease, such as decay of bile canaliculi network and ductular reactions. Organoids incubated with free fatty acids had gene expression signatures similar to those of liver tissues from patients with NASH.

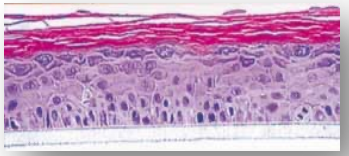
HO will be an excellent platform for dissecting the mechanisms underlying these pathologic aspects of NAFLD that have been largely unexplored because of the lack of relevant human in vitro models.

ALTERNATIVES METHODS VALIDATED: CHEMICAL SAFETY ASSESSMENT

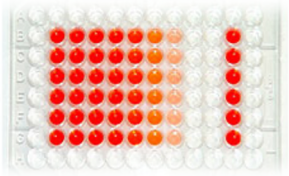
Genotoxicity



Corrosion/irritation



Phototoxicity



Toxicity data generally required in regulatory dossiers of chemical substances

Required toxicity data (by endpoint)	OECD test guideline methods using animals	Animal-free OECD test guideline methods
Acute toxicity (3 routes)	401, 402, 403, 436, 425, 423, 420, 433	
Irritation/corrosion (eye and skin)	404, 405	460, 437, 438, 491, 492, 430, 431,435, 439
Sensitization	406, 429, 442A, 442B	442C, 442D, 442E
Repeated dose toxicity	407, 408, 409, 410, 411, 412, 413, 452	
Genotoxicity	488, 489, 483, 478, 475, 474, 473, 485, 484	471, 490, 487, 476
Carcinogenicity	451, 453	
Reproductive toxicity (fertility and developmental toxicity)	443, 414, 415, 416 (421 and 422 for screening only)	
(Neurotoxicity)[a]	424, 419, 418, (426)	

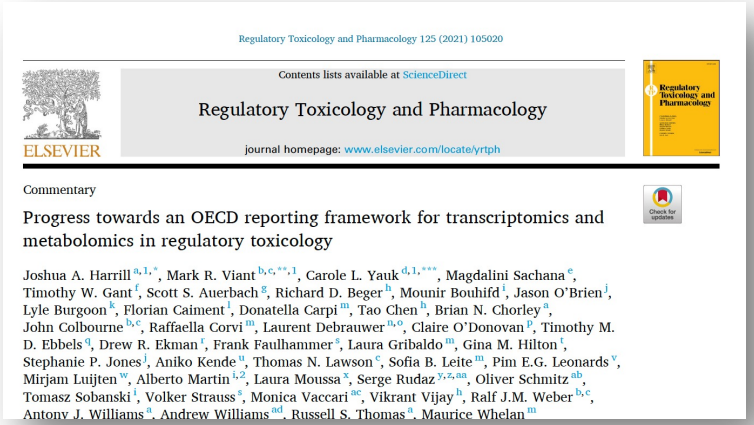
[a] Only few regulations and directives require neurotoxicity data.

Hering al *et al.,* ALTEX, 2019

ADVANTAGES AND LIMITATIONS OF CELL-BASED IN VITRO METHODS

Tools

Omics technologies



Regulatory Toxicology and Pharmacology 125 (2021) 105020

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

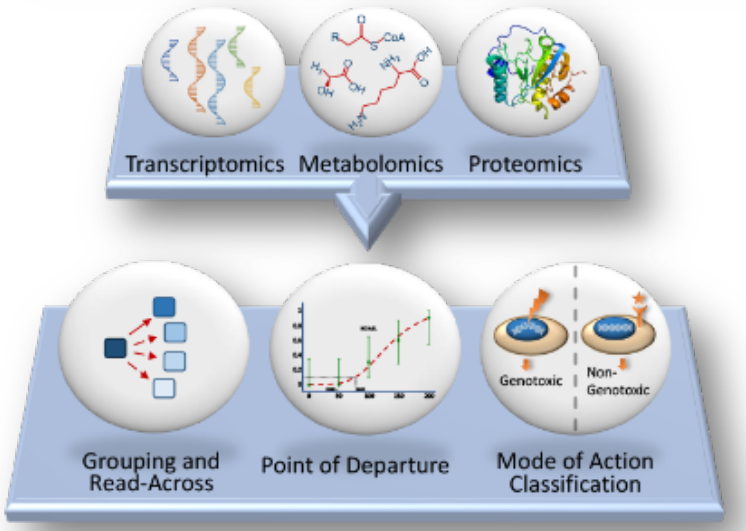
journal homepage: www.elsevier.com/locate/yrtph

ELSEVIER

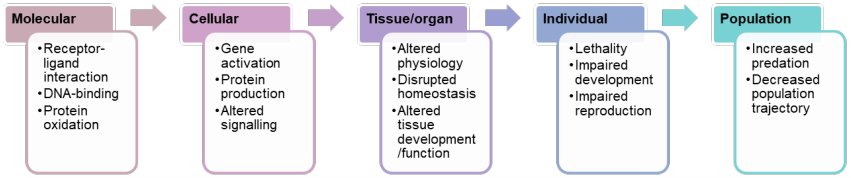
Commentary

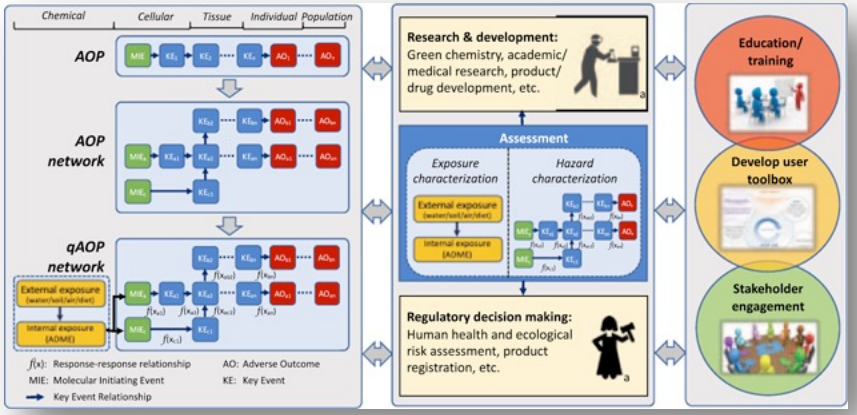
Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology

Joshua A. Harrill [a,1,*], Mark R. Viant [b,c,**,1], Carole L. Yauk [d,1,***], Magdalini Sachana [e], Timothy W. Gant [f], Scott S. Auerbach [g], Richard D. Beger [h], Mounir Bouhifd [i], Jason O'Brien [j], Lyle Burgoon [k], Florian Caiment [l], Donatella Carpi [m], Tao Chen [b], Brian N. Chorley [a], John Colbourne [b,c], Raffaella Corvi [m], Laurent Debrauwer [n,o], Claire O'Donovan [p], Timothy M. D. Ebbels [q], Drew R. Ekman [r], Frank Faulhammer [s], Laura Gribaldo [m], Gina M. Hilton [t], Stephanie P. Jones [j], Aniko Kende [u], Thomas N. Lawson [c], Sofia B. Leite [m], Pim E.G. Leonards [v], Mirjam Luijten [w], Alberto Martin [i,2], Laura Moussa [x], Serge Rudaz [y,z,aa], Oliver Schmitz [ab], Tomasz Sobanski [i], Volker Strauss [x], Monica Vaccari [ac], Vikrant Vijay [h], Ralf J.M. Weber [b,c], Antony J. Williams [a], Andrew Williams [ad], Russell S. Thomas [a], Maurice Whelan [m]



AOP

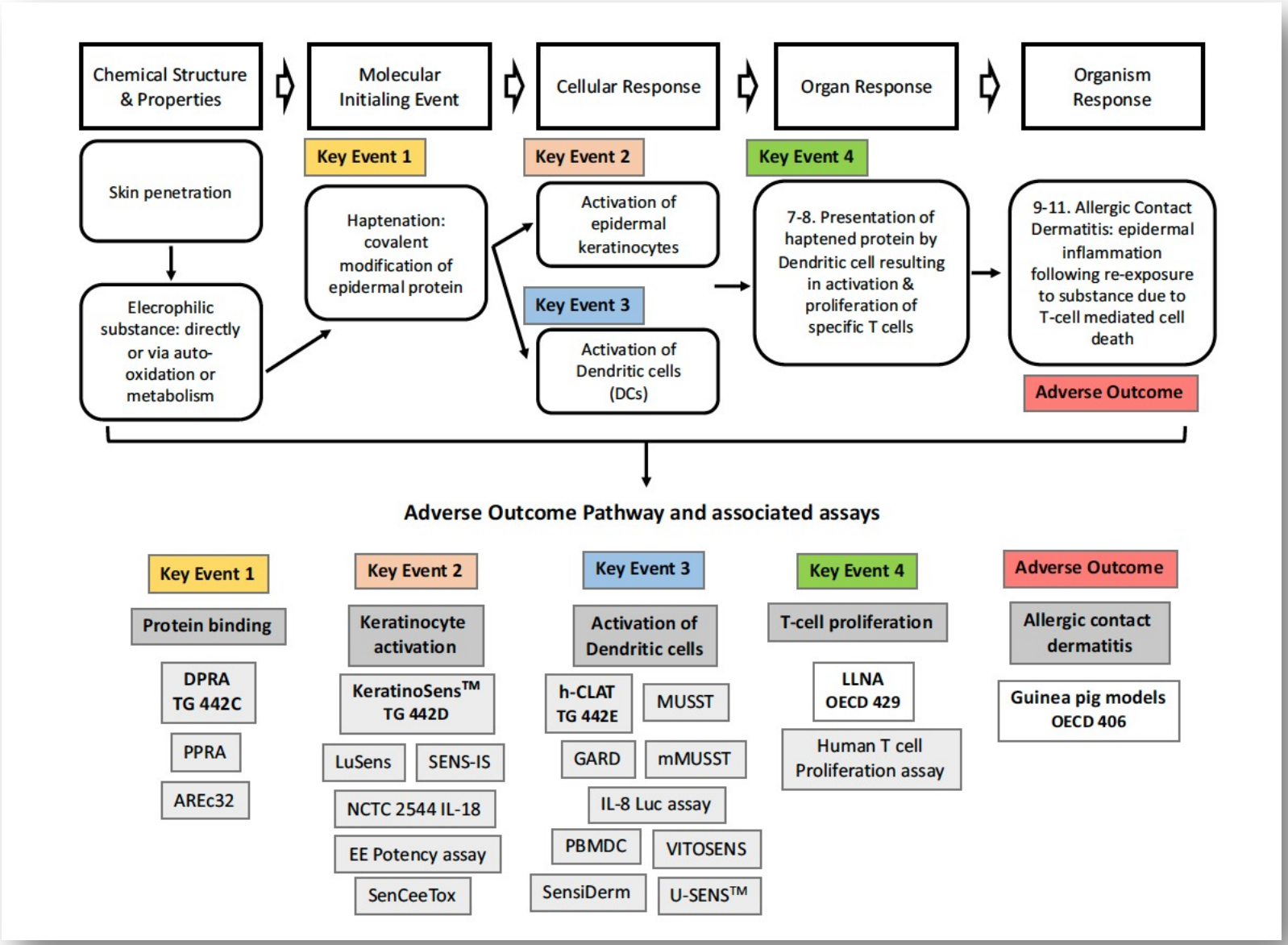




The AOP framework is being increasingly promoted as a useful tool for different applications in **regulatory hazard and risk assessment** of environmental stressors, as well as in research.

SKIN SENSITIZATION AND AOP

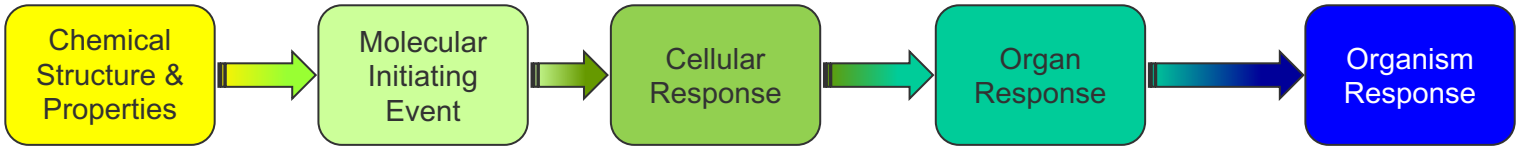
The *7th Amendment to the Cosmetics Directive* prohibited the animal testing for cosmetic products since 2004 and cosmetic ingredients since March 2009.

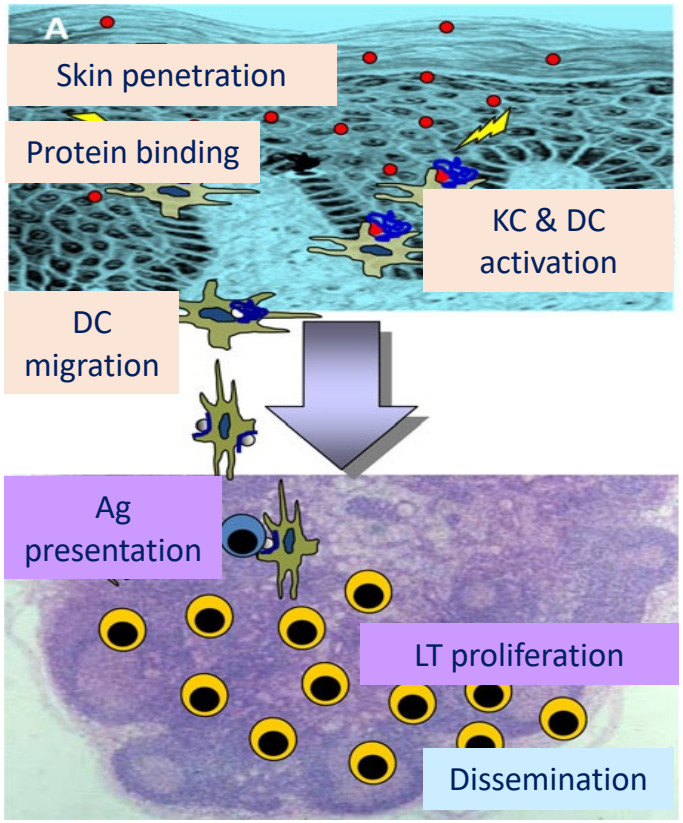


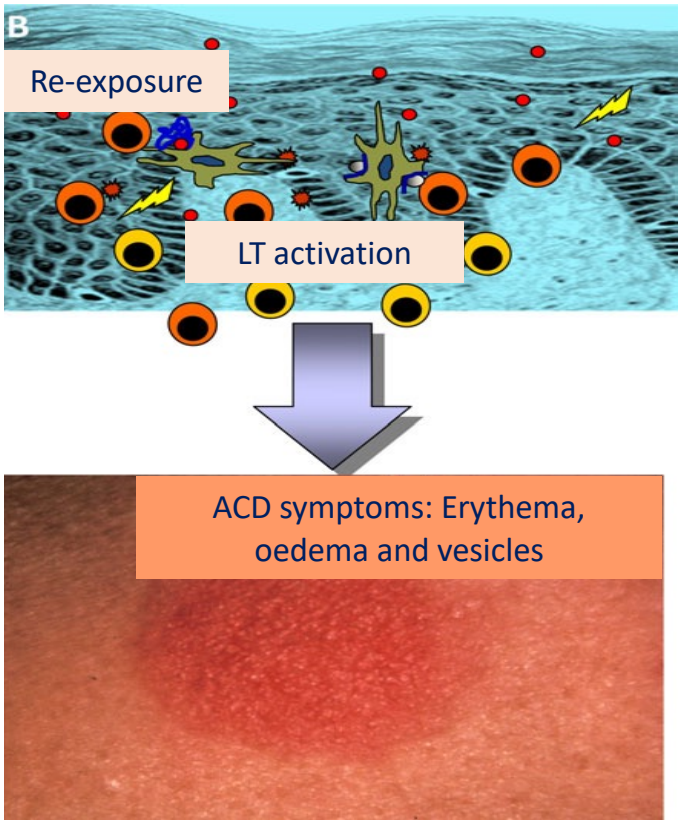
Corsini *at al.*, Archives of Toxicology, 2018

SKIN SENSITIZATION AND KEY EVENTS

AOP (OECD)

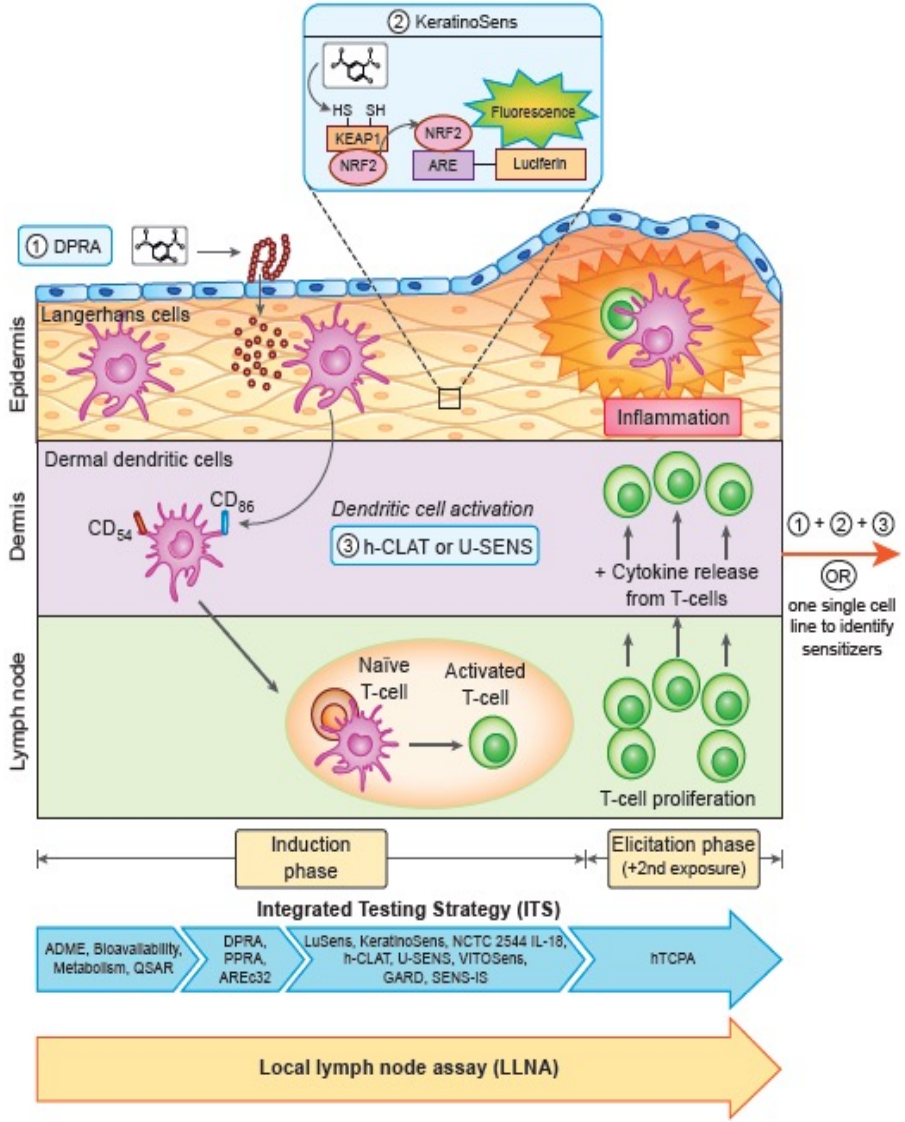






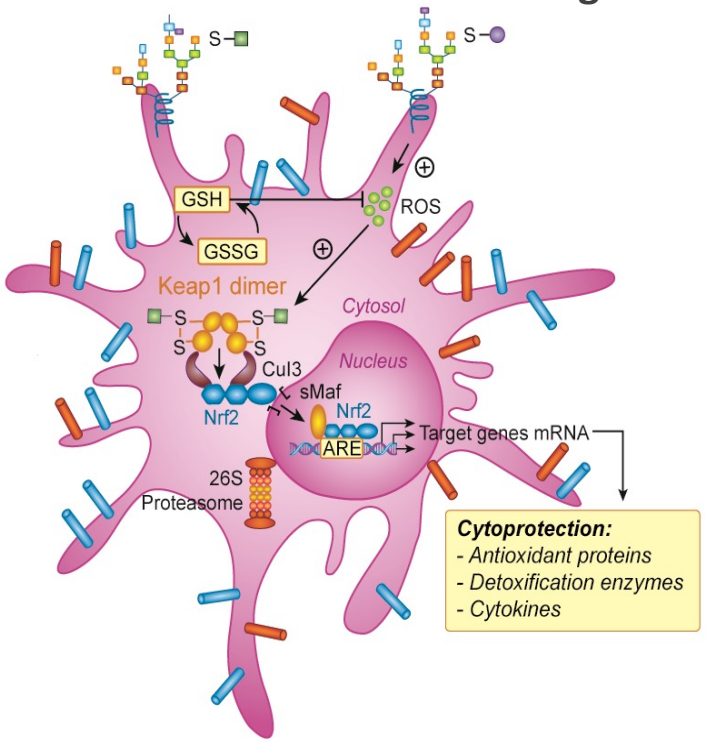
from Kimber *et al.*, Tox. Sciences (2011)

INTEGRATION OF A SET OF ALTERNATIVE TESTS WITHIN THE SAME CELL



KE1- Initial events
KE2- Nrf2 pathway & gene expression
KE3- THP1 phenotype modulation
KE4- LT proliferation

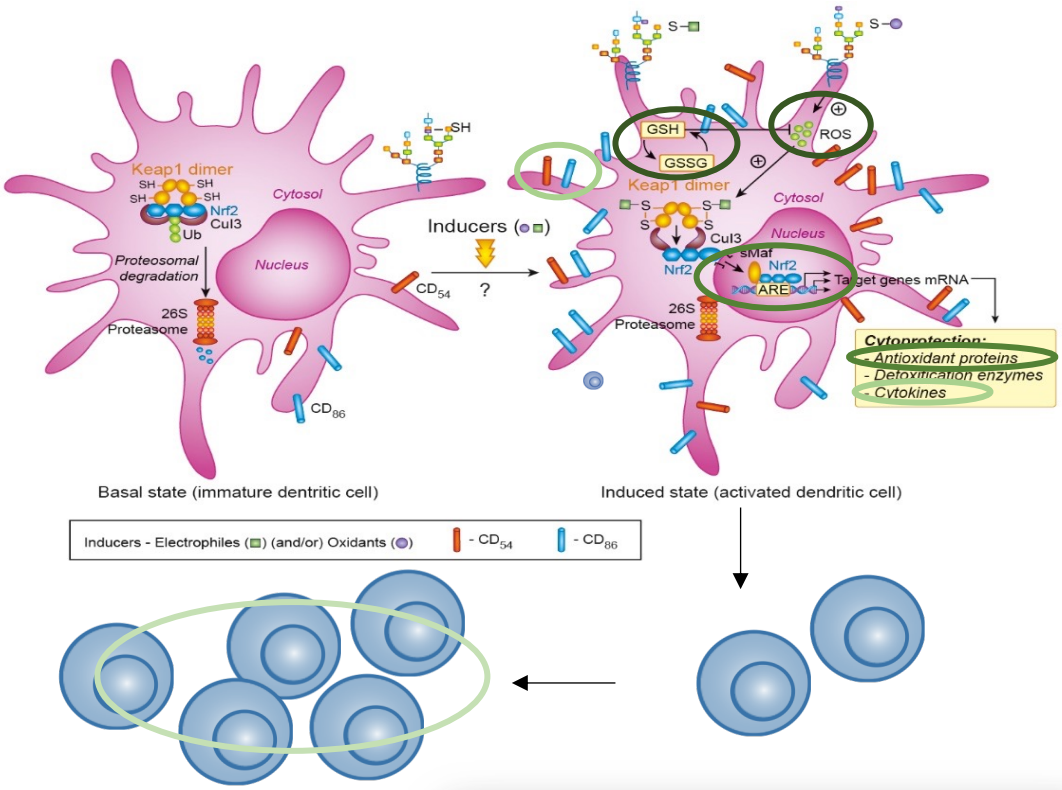
Characterization of the danger

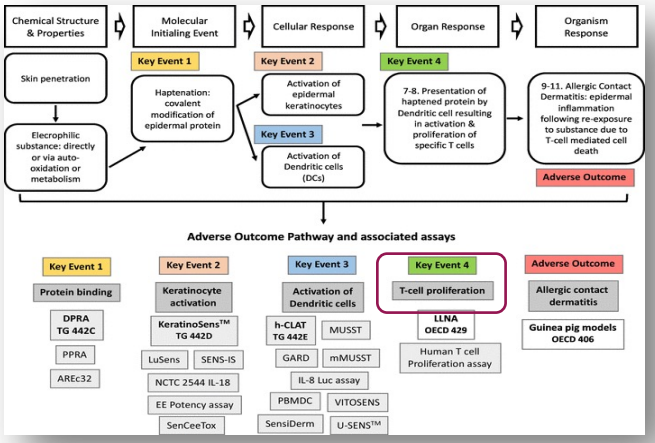


Induced state (activated dendritic cell)

Clouet *at al.,* Archives of Toxicology, 2019

AOP: SKIN SENSITIZATION & KE4





KE1- Initial events

KE2- Nrf2 pathway & gene expression

KE3- Phenotype modulation

KE4- LT proliferation

Clouet *at al.,* Archives of Toxicology, 2019

AOP: SKIN SENSITIZATION

		KE1			KE2	KE3		KE 1		KE 2					KE 3						KE 4
		DPRA				h-CLAT [Kao & Shiseido]		Chemical reactivity		Proteins activity	DNA-binding ELISA	Genes expression on THP-1 (SENS-IS adapted)			Surface markers		Cytokines quantification				
		Cysteine [% peptide remaining]	Lysine [% peptide remaining]	DPRA final result - "1" indicates positive test results	Keratino Sens	CD_{54}	CD_{86}	ROS	GSH depletion	Western blot	Nrf2 TransAM	IRR	SENS-IS	ARE	CD_{54}	CD_{86}	CCL4	IL-8	IL-16	IL-18	T cell proliferation
NON SENSITIZERS	PBS											7	1	2							
	DMSO											6	2	2							
	BZK	97,2	100	0	0							14	4	7							
	t-BHQ											11	1	11							
IRR	SDS	100	100	0	0							13	7	5							
WEAK	RESO	98,4	100	0	0							16	4	12							
	EUG	90,8	80,8	1	0/1							12	2	10							
MODERATE	FAR	92,7	100	0	1							9	8	7							
	GER	100,0	90,0	0	1							7	4	4							
STRONG	CinA	29,4	56,8	1	1							18	6	13							
	MIT	2,1	100	1	1							17	7	11							

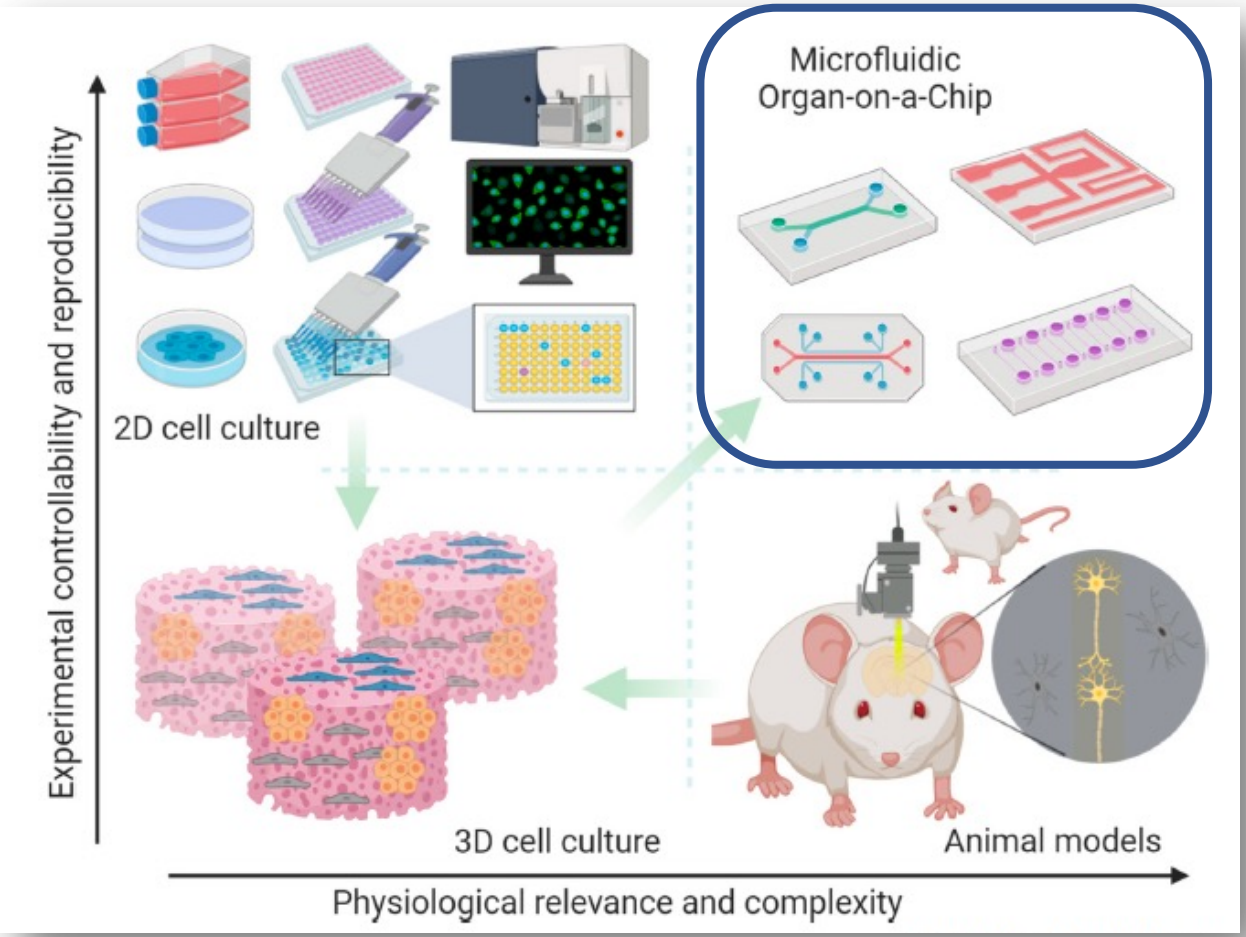
Cut-off: IRR >12 et ARE / SENS-IS ≥7

Negative	Positive	Low Positive (+/-)

Clouet *at al.*, Archives of Toxicology, 2019

TISSUE ENGINEERING: ORGAN-ON-A-CHIP ?

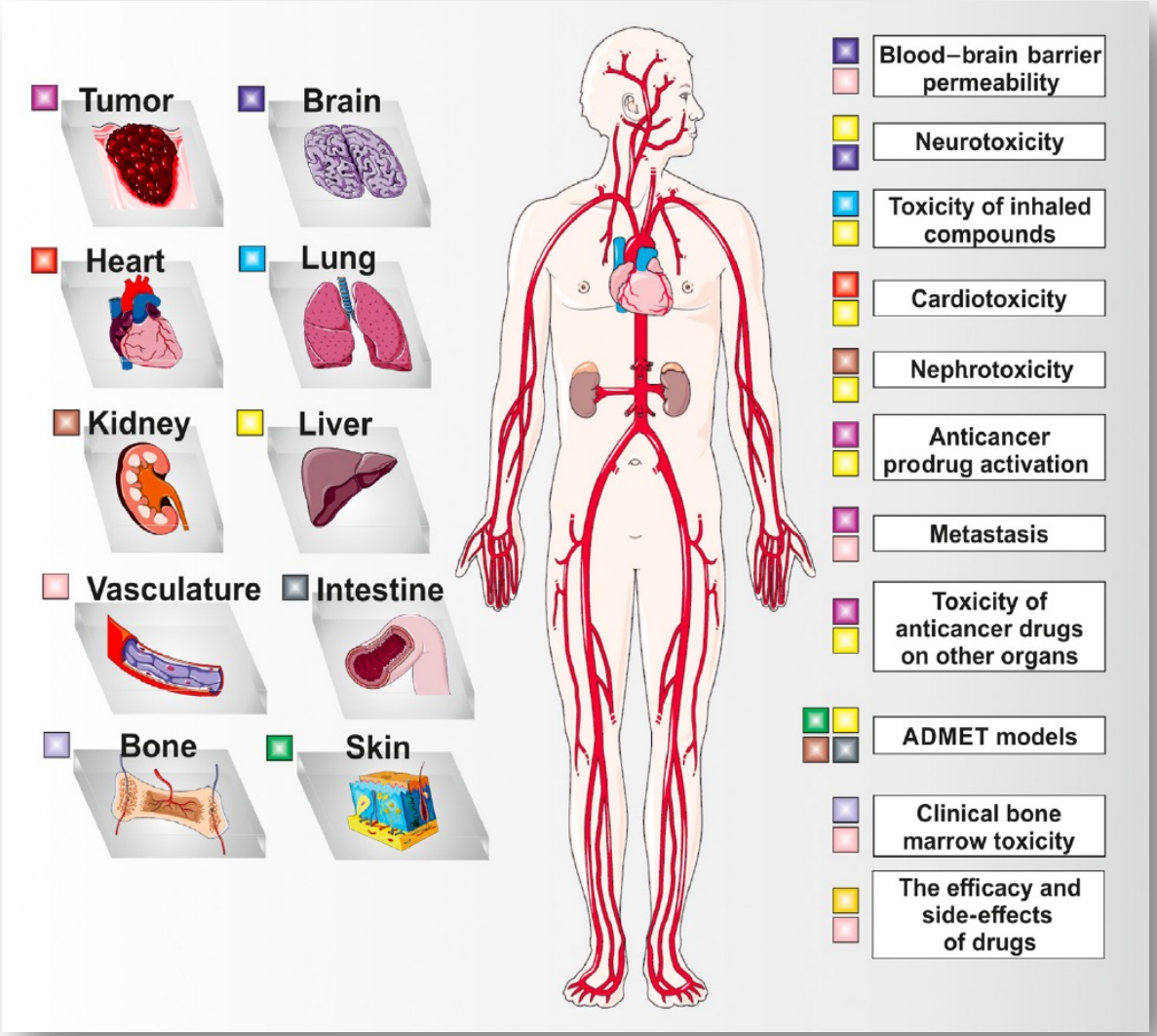
Tissue engineering is the set of techniques used to understand the relationships between the structures and functions of normal and pathological mammalian tissues, in order to develop biological substitutes that can restore, maintain or improve tissue functions.



Ma *et al., Trends in Pharmacology Sciences*, 2021

TISSUE ENGINEERING: ORGAN-ON-A-CHIP ?

Organ-on-a-chip devices aim to mimic the architecture and function of an organ by combining 3D bioengineered constructs .



Picollet-D'haban et al., Trends in Biotechnology, 2021

cross-organ communications & systemic dimension

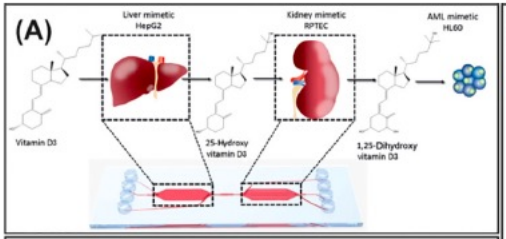
TISSUE ENGINEERING: ORGAN-ON-A-CHIP ?

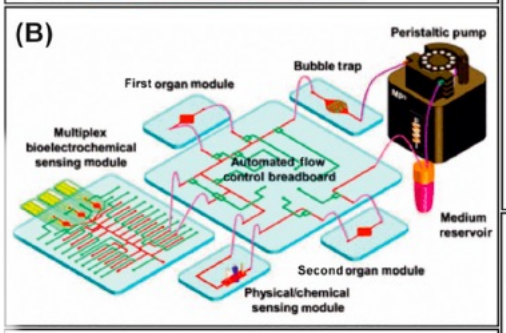
MULTI-ORGAN-ON-A-CHIP (OOC) DEVICES

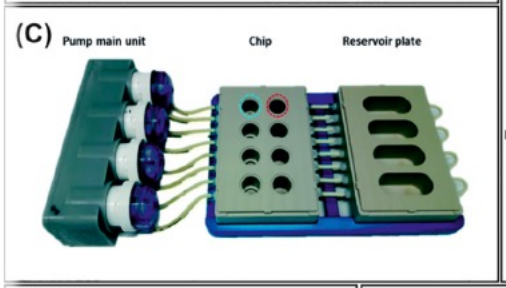
Tissue engineering is the set of techniques used

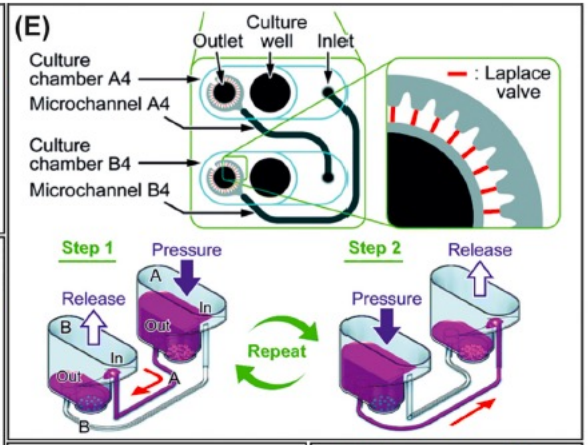
pathological m biological substitutes that can restore, maintain or improve tissue functions.

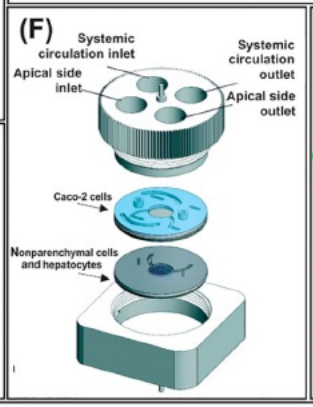
(A) Multicompartment liver–kidney combination in a plate format for studying the metabolism of vitamin D3.

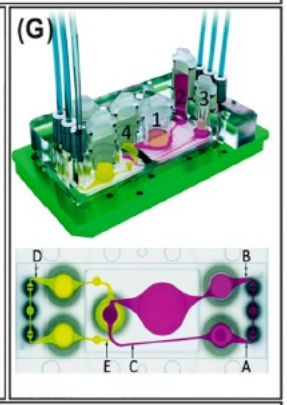




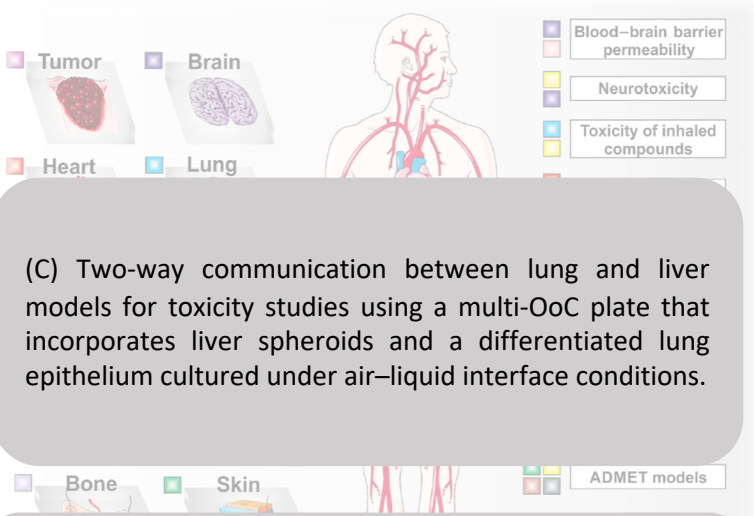




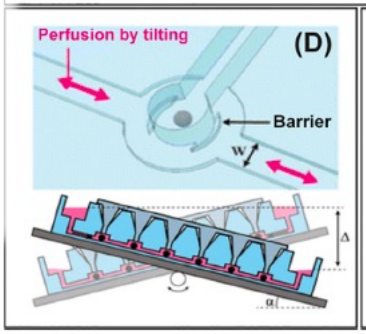


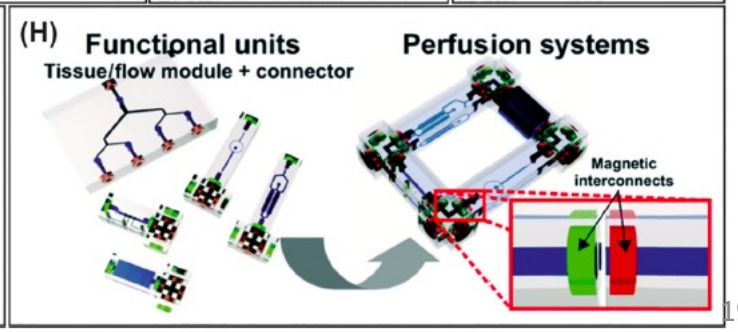


(C) Two-way communication between lung and liver models for toxicity studies using a multi-OoC plate that incorporates liver spheroids and a differentiated lung epithelium cultured under air–liquid interface conditions.



(D) Tilting platform for dynamic medium perfusion in a multi-OoC plate containing spheroid models of liver and colorectal cancer.





Picollet-D'haban et al., Trends in Biotechnology, 2021

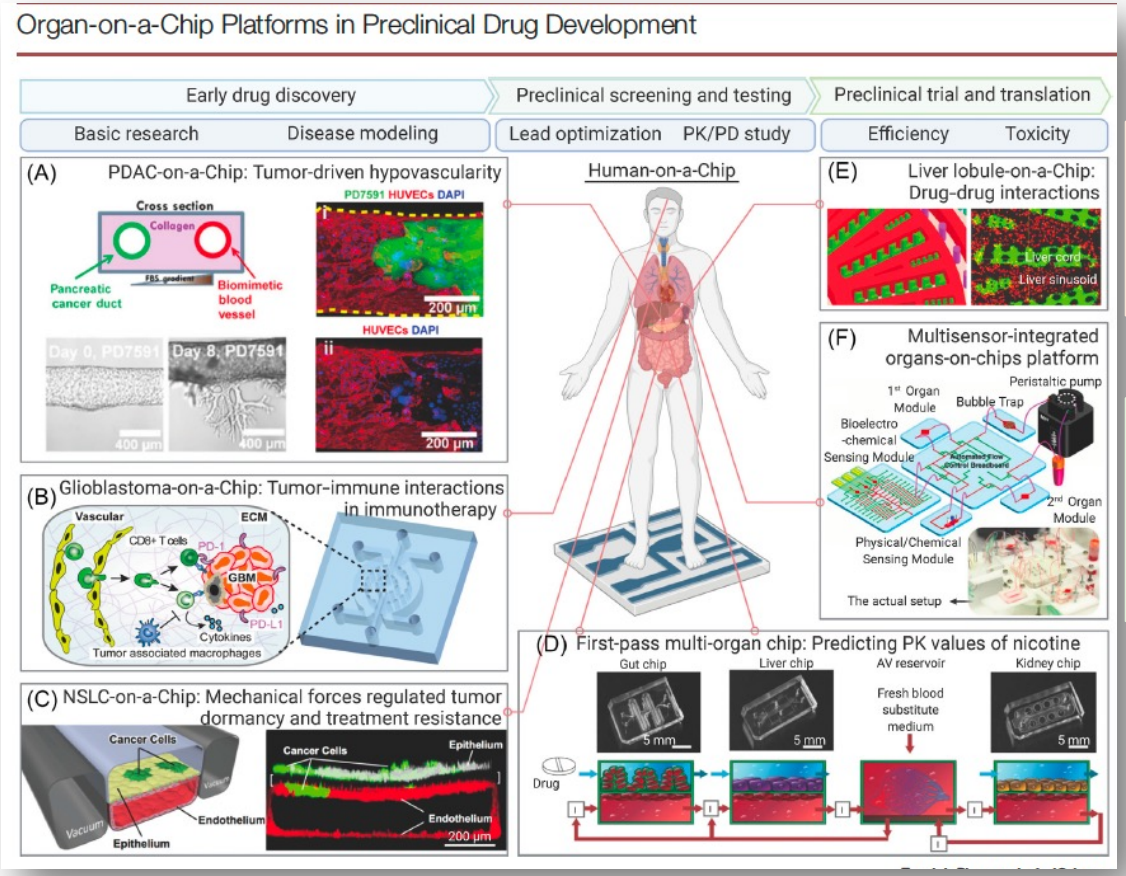
FUTURE ?

ORGAN-ON-A-CHIP: A NEW PARADIGM FOR DRUG DEVELOPMENT?

(A) A PDAC-on-a-Chip with a biomimetic vascular network (HUVECs) and pancreatic cancer duct (PD7591 cells) revealed the Activin-ALK7 pathway as a hypervascularity mechanism for PDAC.

(B) A bioengineered glioblastoma brain tumor model with biomimetic tumor–immune–vascular interactions demonstrated that blockade of immunosuppression contributed by tumor-associated macrophages improved the response to anti-PD-1 immunotherapy.

(C) A NSCLC-microenvironment model study found that mechanical forces during lung breath may promote dormancy and drug resistance of NSCLC cells



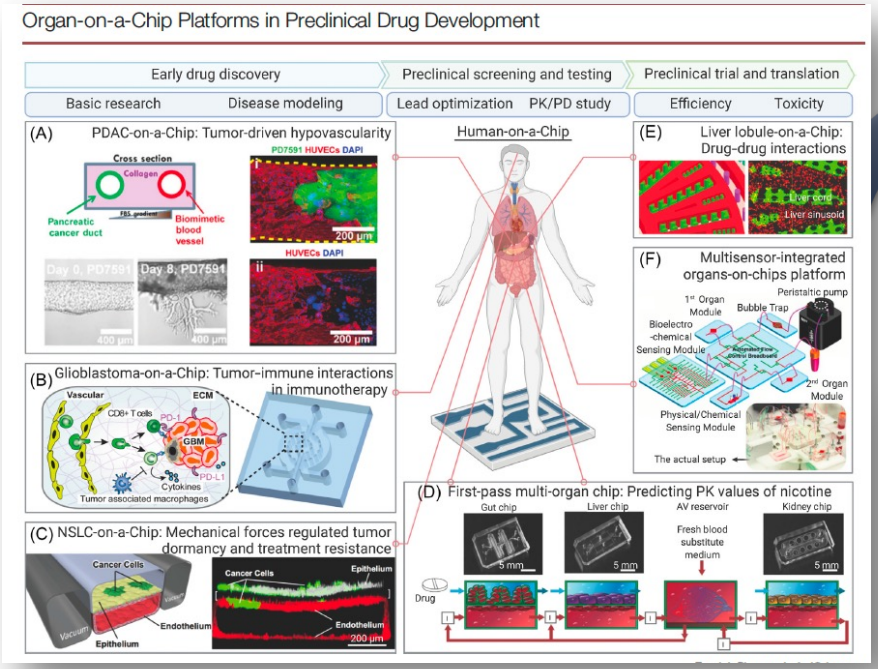
Ma *et al., Trends in Pharmacology Sciences*, 2021

(E) A liver lobule-on-a-chip comprising a liver cord (green) and a liver sinusoid (red) was applied to analyze adverse drug reactions induced by drug-interactions

(F) A multi-organ platform integrated with a multiplex biomarker analysis module was developed to monitor liver toxicity and cardiotoxicity mediated by interorgan metabolism.

PDCA: pancreatic ductal adenocarcinoma
HUVEC: human umbilical vein endothelial cells
NSCLC: non small-cell lung cancer

FUTURE ?



Ma *et al., Trends in Pharmacology Sciences*, 2021

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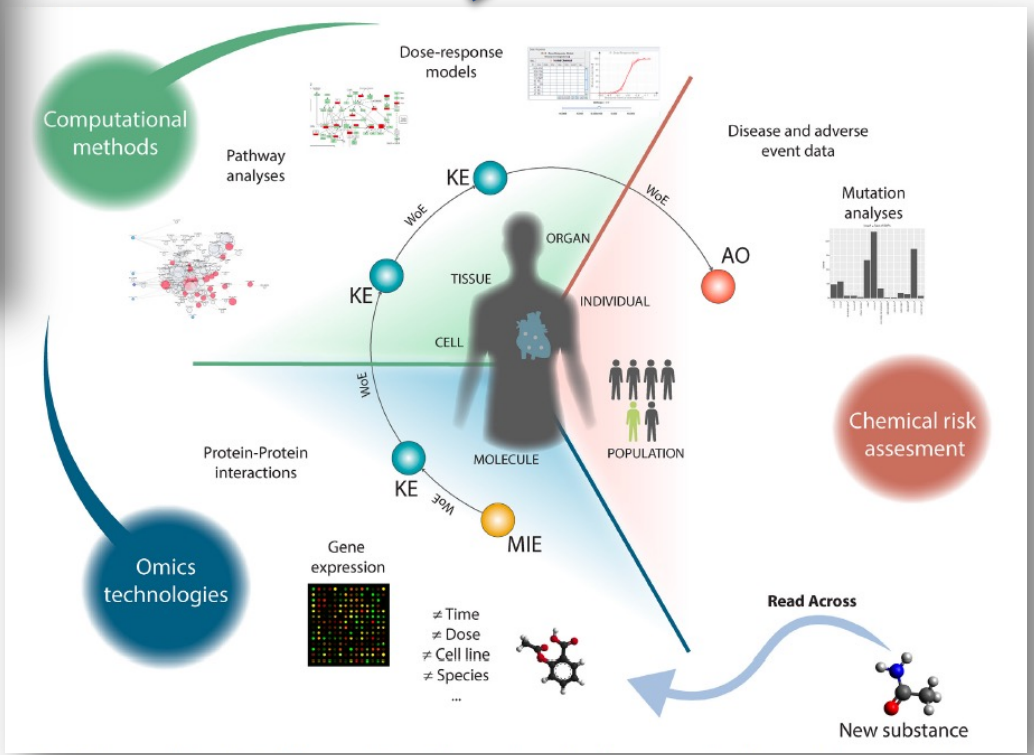

Aguayo-Orozco *et al.*, Current Opinion in Toxicology, 2019


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